OBSERVATIONS ON THE POSSIBILITY OF OBTAINING STREPTOMYCIN-FAST FORMS OF P. PESTIS WITH THE AID OF DNA PREPARATIONS

Translation No. 1709

DECEMBER 1965

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BIOLOGICAL LABORATORIES
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[Following is the translation of an article by L. N. Klassovskiy and L. I. Terentyeva, published in the Russian-language periodical Materialy Nauchnoy Konferentsii po Prirodnoy Ochagovosti i Profilaktike Chumy (Materials from the Scientific Conference on the Natural Focalness and Prophylaxis of Plague), Alma-Ata, Feb. 1963, pages 109--110. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Up until the present time the transfer of a whole number of features, including resistance to antibiotics, has been obtained with the help of DNA preparations (Gochkiss, 1951; Aleksander and Leydi, 1953).

We made an attempt to study the possibility of the transfer of resistance to streptomycin to the plague microbe with the help of a transforming agent. In the literature available to us we were not able to find any data concerning this problem.

In the capacity of DNA strain-donors we used avirulent variants of glycerol positive strains of the plague microbe 1435 and 1125 which are resistant to high (1000 units/ml) concentrations of streptomycin. In the capacity of strain-recipients we used variants of the EV, 1435 and 1125 strains which are sensitive to streptomycin.

The DNA preparations were obtained by the method of Ivory in the modification used by Arkhangelskaya and Terentyeva (1961).

In the first series of two tests we studied the possibility of the realization of transformation under the short-term influence of DNA, obtained from streptomycin fast cells of strains 1435 and 1125, on sensitive variants of the same strains. To the suspension (1 billion of microbial bodies/ml based on optical standards) of cell-recipients we added a preparation of the DNA donor (end concentration of the preparation 1:100 and 1:300), and after a three hour incubation at 28°C we seeded the suspension on Hottinger agar with various concentrations of antibiotic (0, 10, and 1000 units/ml). The seedings were placed in the incubator at 28°C and after three days the number of grown colonies was calculated.

A test was also conducted (strain 1125) in which following the short-term contact with DNA the culture-recipient was exposed to X-rays (dose -- 10800 r, device -- RUM-7) and then tested for sensitivity to the antibiotic.

In the second series of the five tests a streptomycin sensitive variant of strain 1125 was cultivated for two days (at 28°C) in broth, containing DNA (1:10 and 1:100) obtained from a highly resistant variant of the same strain. Then the cultures were seeded on agar plates with a various content of streptomycin and after three days of incubation at 28°C the number of colonies was calculated.

In the third series of experiments (three tests with each of the three test strains) streptomycin sensitive variants of strains EV, 1435 and 1125 were passaged in peptone water, containing DNA from cells of a highly resistant variant of strain 1125. The test cultures were reseeded three times a week (incubation temperature 28°C). All told seven passages were made. The first four reseedings were performed on a medium with a concentration of DNA preparation of 1:2000, and during subsequent passages the content of DNA in the medium was increased up to 1:1000. Following each reseeding the test cultures were tested for sensitivity to streptomycin by the same method as in the other tests (seeding on agar with a various content of antibiotic, three day incubation at 28°C with a subsequent calculation of colonies).

The results of all the tests were evaluated by a comparison of the growth indices (number of colonies) of the test cultures which had been in contact with DNA, and the control cultures which were not subjected to the action of DNA, on agar plates with a various content of streptomycin. In all cases statistically significant differences in the value of the growth indices of the test and control cultures on agar with antibiotic were not detected, that is, under the conditions of our tests we did not succeed in obtaining positive results on the realization of the transformation of the feature of streptomycin resistance in the plague microbe.

It is understood that the results of our tests cannot be evaluated as a final answer to the problem concerning the possibility of obtaining streptomycin fast forms of the plague microbe with the help of DNA. It is very possible that for realizing the phenomenon of transformation additional influences and other conditions are required.